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INHERITABLE MODIFICATION OF THE WATER RELATION IN HIBERNATION OF LEPTINOTARSA DECEM-LINEATA.

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A series of experiments in which *Leptinotarsa decem-lineata* was introduced into the environmental complexes of the deserts at Tucson, Ariz., in nine years of experimentation, have given the results described in this paper; and show how the introduction of an organism from one habitat into another—in these experiments from a mesophytic into desert environment—produce alteration of the water relation in ways that are adaptive in direction and inheritable in character.

The specific result of the experiments concerns the development in the organism, during the period of the experiments, of the capacity to hold water within the tissues so that the intense desiccation of the dry seasons—which are passed in hibernation—does not result in death, and the elimination of the introduced population. The change is adaptive; directly in line with the environmental pressure that is incident upon the population, and in tests is shown to be gametic; in crossing with the normal behaving as a Mendelian dominant; and is not easily reversible.

The materials in the entire series have been pure strains from my laboratory at Chicago; their history, reactions, and genetic composition known for generations before introduction into the Arizona deserts. The materials have at no period in their history been subjected to any experimental operations at Chicago, but are the natural animals, reared under normal conditions, under continuous observation and record. Two strains have been used, No. 99 and No. 100, both derived from nature on the

banks of the Calumet River near Chicago, and therefore characteristic of, and in their activities, adjusted to the conditions of the place of origin.

These stocks have two yearly generations: pass the winter in the adult condition, hibernate in the soil, emerge in the spring, as soon as the temperature is high enough; and begin breeding to produce the first summer generation which matures in July; this first summer generation breeds at once, giving the second summer generation maturing in August or early September. These do not breed, but after feeding undergo preparations for, and enter, hibernation during September or early October, where they remain until the arrival of awakening conditions in the following spring.

In nature this reproductive rhythm is never pure nor regular; many of the overwintering population remain alive, or are late in emergence from hibernation, and are able, therefore, to cross with the first summer generation, giving hybrids between two differing conditions in the rhythm of reproduction, and all materials direct from nature are liable to be in one way or another heterozygous with respect to this rhythm. Pure lines, or stocks in which the rhythm of reproduction was homozygous, have been used in these experiments and no others.

The second summer generation does not mature its gametes, and after feeding undergoes changes preparatory to hibernation that are common to insects; the elimination of waste products and the reduction of the water content of the tissues, lowering the freezing point thereof, so that the animals are not killed by the low temperatures of the winter months, an operation common to many plants and animals in resting conditions. Upon emergence in the spring, or for breeding, this water reduction is rapidly compensated for by water derived from the food and that absorbed by the tissues from the atmopshere or hygroscopic water.

The location chosen for the experiments at the Desert Laboratory of the Carnegie Institution of Washington was on the edge of the broad flood plain of the Santa Cruz River at the foot of Tumamoc Hill, a deposit of gravel and boulder beds with a thick surface layer of adobe soil, of uniform texture and com-

position. All experiments were in cages six feet square and three feet high, with wooden or cement bases that extended into the ground two and one half feet, or below the depth to which the animals burrowed in hibernation. These cages were covered with sixteen mesh pearl wire cloth, which acted to soften the rigors of the desert environment, especially the temperature, rate of evaporation and wind flow, so that the materials while subjected to the rigors of the desert did not receive the whole intensity thereof, but approximately the average conditions.

Throughout the food has been the cultivated potato, as in the original habitat of the materials. All watering was by irrigation about the roots of the plants, and in all respects care was taken to prevent the experiments from having any other than the desert environment about them.

Caged experiments were used instead of isolated locations in the open for two reasons: first, observation and record, as well as protection, could best be maintained in the location and in the arrangements as devised; and second, in caged experiments all selective action by animals that might use these beetles as food was eliminated and the results would, as fully as possible, be those directly due to the physical environmental complex. It developed in the course of the experiments that the arrangements provided not only eliminated this source of complication, but that predaceous enemies, parasites, and epidemics were also absent, no trace of any of them having been discovered, so that as far as I am able to discover, the results are solely the product of the action of the physical environment upon the introduced animals.

The Experiments.

All materials were introduced at the opening of the summer rains, and taken directly from hibernation to Tucson and introduced into the cages, there giving two generations in rapid succession, the second going into hibernation late in the summer, depending upon the environic condition. The following series have given the data for this paper:

T 99, introduced 1908, now in hibernation F_{18}.

T 100, introduced 1909, carried to F_{12} and lost in making repairs.

T 100 A, introduced 1911, now in hibernation in F_{12}.

T 100 B, introduced 1912, now in hibernation in F_{10}.
T 100 C, introduced 1915, now in hibernation in F_4.

Each introduction was taken from the stock at Chicago in the following way: On emergence from hibernation the materials were, of each sex, placed in containers, covered with a cloth, and twenty to twenty-five of each sex removed without inspection, so that there would be no opportunity for selective action on my part, and this parental group of forty to fifty individuals were taken or sent at once to Tucson and placed in the cage prepared with food to receive them. They were then allowed to breed as a population. When the progeny of an introduced group, or if at any other time the population was so large that to breed all was impossible, the same impersonal method of deriving the parental group was resorted to, and it seems highly improbable that with these methods, any selective action could have entered into the series of experiments.

Each introduction has had the same method of derivation from the basal stocks at Chicago, and the same treatment under the conditions of experiment at Tucson, grown as populations, treated as populations, and the results are of the population as a whole, and not of individual lines or mutants.

The series has shown several interesting responses to the introduction into the desert environment, but the most interesting one is the development of a capacity to retain water in the tissues of the second summer, or hibernating generation, instead of eliminating it in preparation for hibernation.

The development of this modification of the animal was first discovered in F_6 of T 99, when a population of over four hundred individuals of the second annual generation was sent to Chicago early in September, 1910 (211 ♂ 234 ♀), placed in a cage in the garden at Chicago, in size and construction like those at Tucson, where they soon entered into hibernation, but absolutely failed to survive the Chicago winter, and were completely eliminated, while a culture of the parent stock in a cage six feet away showed only the normal reaction to that particular winter.

The following year, 1911, tests of this condition were made as follows: T 99 in F_8, of the second annual generation, and T 100 in F_2 of the second annual generation were sent to Chicago

early in September and hibernated in the garden alongside of the original stocks, and a further test was made of T 99 in F_7 in which the first summer generation was crossed as pairs with the normal stock at Chicago, and their F_1 allowed to hibernate in the garden along with the normal and the stocks from Tucson. The results that were derived in the following spring are shown in Table I.

TABLE I.

SHOWING THE RESULTS OF THE TESTING OF THE SURVIVAL DURING HIBERNATION IN THE WINTER AT CHICAGO.

Population Generation, Stock.	Test, Winter of	No.	Hibernated.	Emerged.	Per Cent. Eliminated.	
					Before H.	During H.
T 99, F_8........	1911–12	100♂	87♂	0♂	13	87
		100♀	90♀	0♀	10	90
T 100, F_2	1911–12	100♂	86♂	5♂	14	80
		100♀	89♀	7♀	11	82
*C 99, F_{20}......	1911–12	100♂	91♂	80♂	9	11
		100♀	89♀	84♀	11	16
C 100, F_{12}......	1911–12	100♂	92♂	83♂	8	17
		100♀	87♀	79♀	13	8
F_1 Heterozygotes:						
♂ T 99 × ♀ C 99..........		347♂	317♂	0♂	8.6	91.4
♀ T 99 × ♂ C 99..........		386♀	342♀	0♀	11.4	98.6

* Chicago normal stock.

These tests were made in wire tubes twelve inches in diameter and forty-eight inches long, placed close together, so that all had as far as one can determine the same conditions during the winter. The findings in the emergence from hibernation in May and June, 1912, show, first, that the condition of non-survival in the T 99 materials concerns the entire population, and that in T 100, the same change is coming over the stock but did not involve the entire population, 6.5 per cent. surviving. These surviving individuals of T 100 were placed in breeding cages, where most of them died, two females and one male only taking part in reproduction, giving a first summer generation of eleven males and fifteen females, which died without breeding, although given every condition for reproduction.

The F_1 heterozygous population showed complete elimination, or a total dominance of the condition present in the Tucson parent, which was also present in an equal intensity in both

parents, and equally dominant. On the contrary, the Chicago stocks showed a rather good rate of survival, about the average, and in that the entire test was in a space not over six feet square, with a uniform sandy soil, the result can hardly be attributed to highly localized differences in the winter conditions.

In the summer of 1912 further and more extensive tests were made as follows:

T 99 in F_{10} in the second summer generation to Chicago in September.

T 100 in F_4 in the second summer generation to Chicago in September.

T 100 A in F_2 in the second summer generation to Chicago in September.

The tests for the year comprise the determination of survival in populations that had been at Tucson two, four and ten generations. The results of these tests as determined in the spring of 1913 are given in Table II.

TABLE II.

Population Generation, Stock.	Test, Winter of	No.	Hibernated.	Emerged.	Per Cent. Eliminated.	
					Before H.	During H.
T 99, F_{10}	1912–13	100 ♂	89 ♂	0 ♂	11	89
		100 ♀	92 ♀	0 ♀	8	92
T 100, F_4	1912–13	100 ♂	87 ♂	1 ♂	13	86
		100 ♀	86 ♀	3 ♀	14	83
T 100 A, F_2	1912–13	100 ♂	91 ♂	6 ♂	9	85
		100 ♀	87 ♀	7 ♀	13	80

In T 99 no survivals were found, and the survivals from T 100, although given opportunity to breed, died without issue, and the survivors from T 100 A gave a first summer generation of twenty-six males and thirty-seven females, these a second summer generation of fifty-five males and forty-seven females, of which two males and three females were able to survive hibernation in the following winter, but did not breed after their emergence from hibernation in the spring of 1913.

In the F_1 hybrids in this year (1912), regardless of the direction of the cross, all were eliminated in hibernation, not one appearing in the following spring, again showing the complete dominance of the Tucson trait over the original or normal condition at

Chicago. In the F_2, however, in both crosses survivals occur, and out of 2,137 hibernated, 443 emerged in the spring of 1913. Two random matings were made from these, of twenty males and twenty females, and large populations grown for the second summer generation and hibernated under the usual conditions, gave in the spring of 1914 the following results:

	Hibernated.		Emerged.	
	Males.	Females.	Males.	Females.
Mating *A*.	861	905	672	689
Mating *B*.	919	945	792	807

These in all ways were not to be distinguished from the normal stocks, and allowing for the normal elimination incident to hibernation under the conditions provided, which averages in my garden about twenty per cent., the experiences of the experiment suggest that we have in the F_2 popuulation of 1912

	Completely Eliminated. 1 DD. + 2 Dr.	Surviving. 1 rr.
Observed	1,694	443
Expected	1,602.75	534.25 if a normal monohybrid.

In that the normal elimination in hibernation of the Chicago stock runs about twenty per cent., the eliminated individuals in this test of F_2 are surely the combined DD and Dr portions of the population, plus the non-distinguishable dead individuals of the (rr) portion of the array.

This method of experimentation and testing has been continued in each year to the date of writing, the results of which show that the condition of non-capacity to survive the winter at Chicago is a gradually increasing product of the populations at Tucson, and that the behavior in crossing of the Tucson characteristic is uniformly that of a Mendelian dominant, the normal Chicago condition appearing in F_2 in numbers that are as close to expectation as could be expected.

Since the discovery of this condition in T 99, all of the introductions of *L. decem-lineata* have been tested in the hibernating generation with respect to their capacity to survive the winter conditions of the habitat from which the stock came. The results of these tests are presented in Tables III., IV., V. and VI.

TABLE III.

SHOWING IN CULTURE T 100, THE COMPLETE FAILURE OF SURVIVAL IN THE POPULATIONS OF THIS CULTURE AFTER SIX GENERATIONS IN THE DESERTS OF TUCSON.

Population Generation.	Test Winter of	Number Taken.	Hibernated.	Emerged.	Per Cent. Eliminated.	
					Before H.	During H.
F6	1911–12	100 ♂	87 ♂	0 ♂	13	87
		100 ♀	90 ♀	0 ♀	10	90
F8	1912–13	50 ♂	47 ♂	0 ♂	6	94
		50 ♀	44 ♀	0 ♀	12	88
F10	1913–14	25 ♂	23 ♂	0 ♂	8	92
		25 ♀	22 ♀	0 ♀	12	88
F12	1914–15	50 ♂	44 ♂	0 ♂	12	88
		40 ♀	39 ♀	0 ♀	22	78

TABLE IV.

SHOWING THE RESULTS OF THE TESTS IN CULTURE T 100 A, IN WHICH ELIMINATION DURING HIBERNATION IS SHOWN TO INCREASE PROGRESSIVELY, ENDING IN A CONDITION IN WHICH SURVIVAL OF THE WINTER AT CHICAGO IS NO LONGER POSSIBLE FOR THE POPULATIONS IN THIS CULTURE.

Population Generation.	Test Winter of	Number Taken.	Hibernated.	Emerged.	Per Cent. Eliminated.	
					Before H.	During H.
F2	1911–12	100 ♂	91 ♀	6 ♂	9	85
		100 ♀	87 ♀	7 ♀	13	80
F4	1912–13	100 ♂	84 ♂	1 ♂	16	83
		100 ♀	87 ♀	3 ♀	13	84
F6	1913–14	50 ♂	44 ♂	0 ♂	12	88
		50 ♀	45 ♀	0 ♀	10	90
F8	1914–15	100 ♂	89 ♂	0 ♂	11	89
		100 ♀	93 ♀	0 ♀	7	93

TABLE V.

SHOWING THE SURVIVAL TESTS MADE IN THE SECOND, FOURTH, SIXTH AND EIGHTH GENERATIONS OF THE CULTURE T 100 B, IN WHICH THE PROGRESSIVE INCREASE IN THE ELIMINATION DURING HIBERNATION FROM THE SECOND TO THE EIGHTH GENERATION, RESULTS IN COMPLETE ELIMINATION IN THE LAST TWO TESTS MADE IN THIS SERIES.

Population Generation.	Test Winter of	Number Taken.	Hibernated.	Emerged.	Per Cent. Eliminated.	
					Before H.	During H.
F2	1912–13	100 ♂	89 ♂	5 ♂	11	84
		100 ♀	86 ♀	7 ♀	14	79
F4	1913–14	100 ♂	91 ♂	2 ♂	9	89
		100 ♀	90 ♀	3 ♀	10	87
F6	1914–15	100 ♂	86 ♂	0 ♂	14	86
		100 ♀	87 ♀	1 ♀ died	13	86
F8	1915–16	50 ♂	45 ♂	0 ♂	10	90
		50 ♀	42 ♀	0 ♀	16	84

Table VI.

Showing the Test Made upon Culture T 100 C, Introduced into the Tucson Deserts in June, 1915, where it Reproduced, the Sample of the Second Summer Generation, Taken to Chicago, in September, and Subjected to the Survival Test in the Winter of 1915–16.

Population Generation.	Test Winter of	Number Taken.	Hibernated.	Emerged.	Per Cent. Eliminated.	
					Before H.	During H.
F_2............	1915–16	50♂ 59♀	46♂ 44♀	4♂ 6♀	8 12	84 76

These tests shown in the above tables have been made in the wire tubes, placed close together, and having as far as could be determined the same conditions during a given winter. Undoubtedly the testing in tubes favors the culture in the elimination of possible enemies in the soil, and in fact, the survival of the normal stocks in the tubes at Chicago is higher than in large open cages, so that any action of the tube is favorable to survival rather than otherwise.

In all of the introductions precisely the same action is going on, and in all of the tests the same result shows at about the same rate of progression, namely, that the capacity to survive the winter diminishes progressively as the number of generations increases in which the stocks have lived under the conditions of the Arizona deserts. The greatest change comes between introduction and the hibernation of the first year.

At Chicago for some years I have made routine tests of certain of the activities concerned in hibernation, in that this period of passing of the unfavorable portion of the year in their habitat is a season of possible elimination, of possible selective action, therefore, one of possible effectiveness in the evolution of the population. In Table VII. I have shown a series of tests made in the stock C 100 from the eighth to the eighteenth generations, inclusive. These were all made in wire tubes sunk in the ground, under similar conditions of soil, moisture and climatic exposure.

The tests in Table VII. show that in the preparation for hibernation, on the average, eleven per cent., of the tested materials are not able to complete the preparations and do not enter into hibernation. These will be found dead upon the surface of

Table VII.

Showing in the Original Culture of *L. decem-lineata* No. 100, at Chicago. Tests Made to Determine the Elimination in the Hibernating Population, in Preparation for Hibernation, the Number that Hibernated, and the Percentage Eliminated During Hibernation, with the Percentage of Survivals in the Following Spring.

Stock Culture No. 100, Chicago.

Population Generation.	Test Winter of	Number Taken	Hibernated.	Emerged.	Per Cent. Eliminated.	
					Before H.	During H.
F_8	1910–11	100♂	91♂	83♂	9	8
		100♀	87♀	80♀	13	7
F_{10}	1911–12	100♂	96♂	82♂	4	14
		100♀	83♀	79♀	17	4
F_{12}	1912–13	100♂	94♂	80♂	6	14
		100♀	88♀	77♀	12	11
F_{14}	1913–14	200♂	181♂	160♂	9.5	10.5
		200♀	173♀	164♀	13.5	5
F_{16}	1914–15	100♂	88♂	71♂	12	7
		100♀	90♀	67♀	10	13
F_{18}	1915–16	100♂	83♂	77♂	17	6
		100♀	91♀	74♀	9	15
Totals		700♂	633♂	553♂	av. 9.583+	av. 9.916+
		700♀	612♀	541♀	av. 12.416+	av. 9.166+
		1,400	1,245	1,094	av. 11	av. 9.541+
Total elimination in connection with hibernation, av.					20.54+	

the ground in the tube, but what the cause of the elimination is has not been determined, and may possibly not be possible of determination as the population will not breed until after hibernation and it is therefore impossible to attempt to get a culture of these individuals for further testing, and further there are no indications that have been detected that they are in any way different from the remainder of the tested population. Moreover the sample for testing has always been drawn from the stock culture, in an absolutely impersonal manner and at random so that the elimination may for the present be regarded as a chance elimination in the preparations for hibernation, of about the same value in each hibernating generation, therefore of no probable modifying action upon the population.

From the same table it appears that during hibernation there is on the average an elimination of nine and one half per cent.

of the tested populations, or a total elimination incident to hibernation of the second summer generation of these animals of about twenty per cent. of the population, about eighty per cent. emerging in the following spring. In nature, however, the elimination is greater, due no doubt to agencies that are eliminated under the conditions of these tests, which completely cut off all the losses that might come from burrowing animals that might use them as food, and probably other agencies as well.

In Table VIII. are given the combined results of the testing of the introduced cultures at Tucson, respecting their ability to survive the hibernating season of their native habitat.

Table VIII.

Showing the Combined Results of the Testing of the Cultures T 99, T 100, T 100 A, T 100 B, T 100 C, from the Second to and Inclusive of the Sixteenth Generations. It Should be Noted that the Percentage of Elimination before Hibernation, Remains About at the Average for the Species as Shown in Table but that the Elimination During Hibernation Increases, until About the Sixth Generation, When it Becomes Total, and Remains so to the End of the Series to Date.

Population.	No.	Hibernated.	Emerged.	Per Cent. Eliminated. Before Hib.	Per Cent. Eliminated. During Hib.	Per Cent. of Population Surviving.
F_2 T 100 A.... T 100 B.... T 100 C....	250♂ 250♀	226♂ 217♀	15♂ 20♀	9.33+ 12.66+	84.33+ 78.66+	7.5
F_4 T 1100 A.... T 100 B....	200♂ 200♀	175♂ 177♀	3♂ 6♀	12.5 11.5	86 85.5	2.25
F_6 T 100...... T 100 A.... T 100 B....	250♂ 250♀	217♂ 222♀	9♂ 1♀ died	13 11	87 88.66+	.20
F_8 T 99...... T 100...... T 100 A.... T 100 B....	300♂ 300♀	268♂ 270♀	0♂ 0♀	10 11	90 89	0
F_{10} T 99...... T 100......	125♂ 125♀	112♂ 114♀	0♂ 0♀	9.5 10	90.5 90	0
F_{12} T 99...... T 100......	200♂ 200♀	177♂ 178♀	0♂ 0♀	12 11	88 89	0
F_{14} T 99......	100♂ 100♀	89♂ 93♀	0♂ 0♀	11 7	89 93	0
F_{16} T 99......	100♂ 100♀	86♂ 90♀	0♂ 0♀	14 10	86 90	0

Comparison of the data in Tables VII. and VIII. shows conclusively: First, that the elimination in the preparation for

hibernation remains at about a constant figure in both the introduced materials at Tucson and in the original materials that have lived continuously at Chicago; second, that the elimination during hibernation takes a great increase in all the second generations tested, from 9.5 per cent. to over 80 per cent., and then in the next following years increases slowly until the sixth, when elimination is complete and continues to be so, no capacity to resist the Chicago winter conditions appearing in any of the Tucson materials in the later generations.

Beyond question, therefore, some change has taken place as a consequence of the introduction of *L. decem-lineata* from the

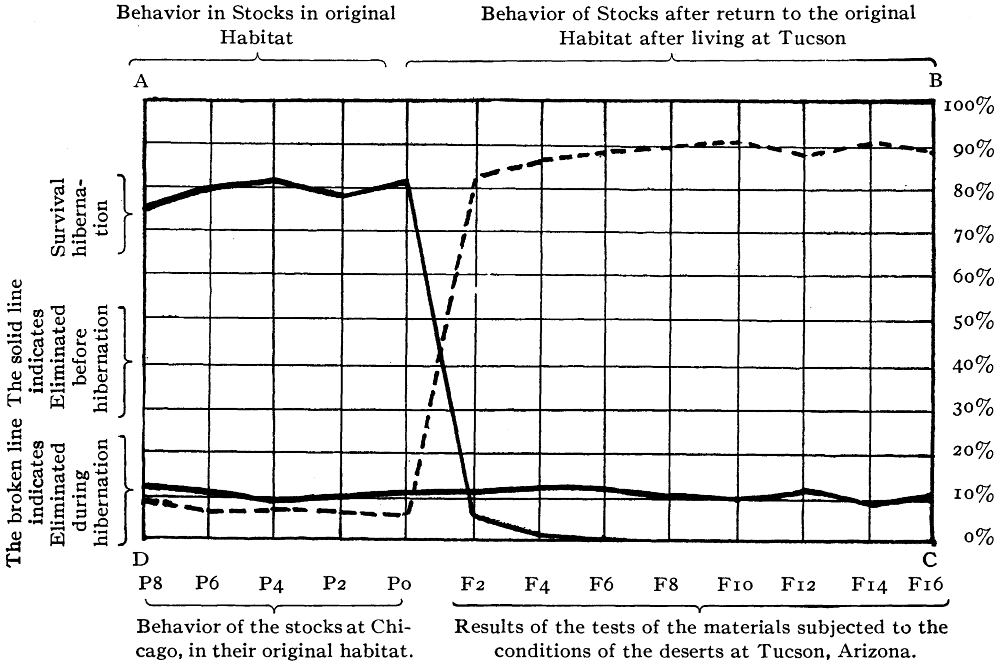

FIG. 1. Graph showing the curve of survivals from hibernation, of elimination in preparation for hibernation, and of elimination during hibernation.

habitat at Chicago into the deserts at Tucson, in the change from a moist habitat into one of intense desiccation during the resting season, which is passed in hibernation. This change is made graphic in Fig. 1, showing the line of elimination in the preparation for hibernation, remaining constant throughout the

series, the sharp changes in the line of survival of hibernation and in elimination during hibernation that occur between the introduction and the first hibernating period, at the end of the second generation.

In hibernation temperature and humidity in the soil are the two chief factors concerned with these animals, and their action upon the organisms is difficult of isolation. In the two environments chosen for the experiments, the conspicuous difference is one of humidity, not of temperature; soil conditions at Chicago during the period of hibernation having a high and rather constant water content, whereas at Tucson the relation is one of continuously diminishing humidity. At the close of the summer rains at Tucson, the intense desiccation accompanying the high temperatures of the dry after summer, rapidly lowers the water content in the soils until the onset of the winter rains in December, and again in the dry fore summer from the end of the winter rains in March to the beginning of the summer rains in July, the water content is progressively lowered until at the end of the period it may be as low as 5 per cent. or even less in the upper layers of the soil. It is this latter period in which the most intensive elimination of the cultures at Tucson takes place.

In desert organisms, in general, some mechanism is present by which the water content of the resting organisms is conserved during the dry and resting periods in the year, otherwise the plants or animals are not able to survive the hostile conditions of these rigorous portions of the year that are so characteristic of every desert environmental complex, and which may be prolonged, irregular, and highly variable in their manifestation in different years. Consequently, surviving desert types must meet, not only the average extreme and hostile conditions in their existence, but irregular and extreme manifestations of the environic complexes, often of prolonged duration. In other words, a desert environment is not only a most rigorous one, but also about the most variable habitat that an organism has to meet.

The response found in these experiments at Tucson suggests at once that the alteration is one of water relations, one in

which the capacity to hold the water in the hibernating generation has developed. Tests of the capacity to hold the water in the tissues against desiccation have been made in the different series of experiments, with rather striking results.

These tests are made by taking about equal weights of one of the Tucson cultures from the generation that is hibernating, and from the cultures at Chicago, enclosing them in small

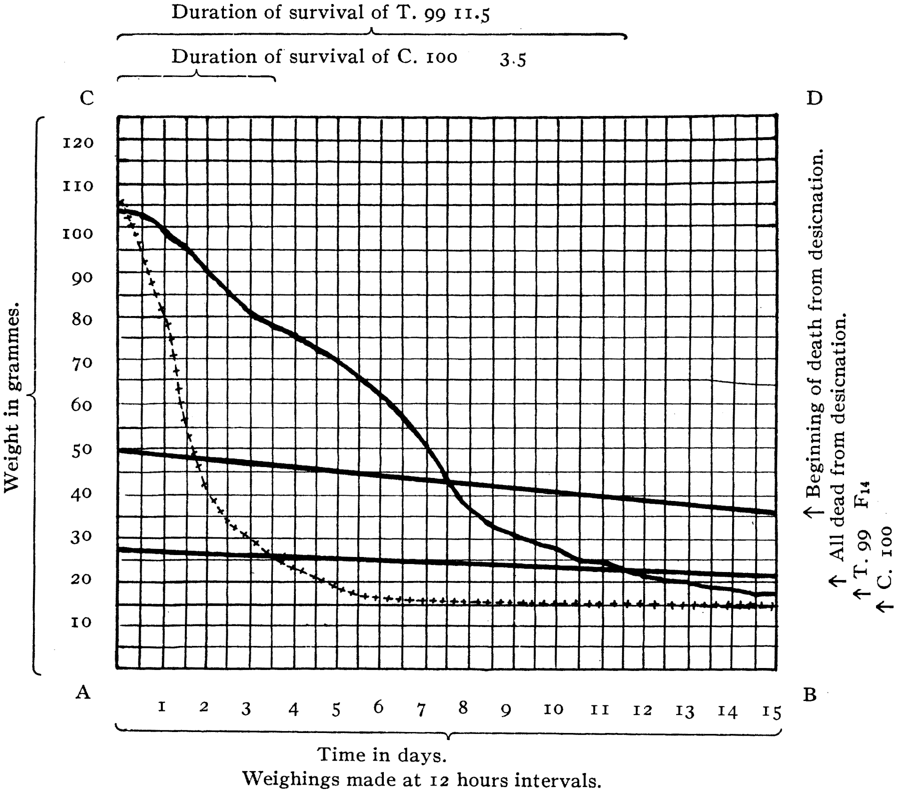

FIG. 2.

wire cloth containers, the two, then subjected to intense desiccation by the passage of a current of dehydrated air at a uniform rate through the materials until death has resulted, and the materials attained a uniform dry weight, weighings being taken every twelve hours.

These tests show that the Tucson materials, especially after

the sixth generation at Tucson, have constantly the capacity to hold onto the water content in their tissues, are more resistant to desiccation, and in every way are different in their reactions to the water loss than are the same materials that have lived at Chicago continuously. In Fig. 2 I have plotted the results of one of these dual tests made upon T 99 in the fourteenth generation of its existence at Tucson, and the stock C 100 from Chicago.

The curve of response of the materials at Chicago shows a rapid water loss in the first day, the onset of death from desiccation by the end of the second, and the death of all in this test at three and one half days, with the attainment of a constant dry weight under the conditions at about the sixth day. In the sample from Tucson, however, the curve of response to desiccation shows a much less rapid loss, the first deaths not occurring until the seventh day and total death not until the twelfth day, and dry weight constancy about the fifteenth, but not as low as in the culture of Chicago materials. The temperature in this test was constantly 20° C., the temperature used in all such testings.

There is a very obvious difference in the ability of the two sets of organisms to let go of the water in the tissues under-desiccating conditions in the environment. In these tests the conditions were most severe, with absolutely dry and rapid passage of air, in all respects immensely more severe than are ever obtained in any desert complex during hibernation, as the animals are then protected in the ground and desiccation is slow and gradual. However, the desert adjusted group shows that it has the ability to hold the water, or less capacity to let the water loose from its tissues when subjected to the pull of an intensely dry environic condition, and this one difference would decide the fate of either set of materials in different environments.

The eliminations in the survival tests made at Chicago of the samples from the Tucson stocks, are clearly due to their not being able to let go of the water in their tissues with sufficient rapidity to keep the freezing point below the temperature of the soil, consequently they are actually frozen by the decreasing temperatures accompanying the onset of the northern winter, and examination of test cultures made for the purpose at Chicago shows

that few of the individuals in the test survive beyond December first, and none have been found alive by the end of the same month. Further many of the individuals seen in these examinations when recovered from the ground show frost crystals within the tissues, and extensive disruptive actions thereof.

Repeated tests in the different cultures at Tucson, at early and late generations during their history, show that the retention of water in the tissues against the influences of a strongly desiccating environic complex gives a series of findings that are in close accord with the data derived from the survival tests of the same materials at Chicago; a sharp change in this capacity between introduction and the first period of hibernation, and thereafter a slower increase through the following hibernation periods, until the full capacity is developed, at about the sixth generation after introduction.

From all the different aspects in which this response to the changed environic complex is viewed, it gives only the one conclusion, that there has resulted from the sharp change in the living conditions the development of a capacity to hold water and not lose water in the presence of conditions in the surroundings, which in the original materials causes rapid loss of water with eventual death. The modification is, therefore, of a direct adaptive nature, in direction and kind to meet the needs of the new environment.

What the alteration has actually been in the mechanism of the organism, I do not know. Thus far I have not been able to detect any anatomical modifications, or changes of a cytological character that have taken place. Water loss in these animals is through the dermal glandular secretions and respiratory activity. As far as I can determine, there is no decrease in the dermal glands that are present, either in size or number, or any alteration in their distribution, although I have searched diligently therefor. Moreover, there are no indications of a thicker cell wall, either in the hypodermis or of any increased thickness in the cuticular linings of the tracheal tubes.

Whether it is due to changes in the permeability of the cell membranes or to changes in the colloidal contents of the cells is a matter of opinion, as I have no evidence that supports

either view with any certainty, and accurate determination of either of these is difficult, if not quite impossible. Regardless of what the actual change may consist of, it is an evolutionary modification, that is directly adaptive in response to altered conditions of life, and as such is well worth careful examination and further investigation.

Perchance it may seem that it is easy to arrive at results of this kind. To prevent any such misapprehension, the experiences in the introduction of other species into the same environic complexes may be cited. During the years in which these experiments with *L. decem-lineata* have been in progress, there have been introduced with the same care and persistency *L. signaticollis*, *diversa*, *undecimlineata*, *panamensis*, *multitæniata*, *oblongata*, *haldemani*, *juncta*, and *dilecta*, representing a diversity of environmental origins and adjustments to temperature and moisture during the hibernation period, and in none of them has anything like the result described in *decem-lineata* been found. The closest approximations thereto are in the species *multitæniata*, *oblongata*, and *signaticollis*, all of which live in haibtats having rather high rates of desiccation during the resting season, and survival in the case of these introductions has been irregular, and the product of favorable conditions in the particular winter, rather than any alteration resulting from the introduction. All of these species, further, live in areas in which the temperature in the resting season is fairly high, so that there is no necessity for the reduction of the water content to prevent elimination by frost action.

Inheritance.

It was shown in connection with some of the earlier tests that the alteration when crossed with the normal condition at Chicago, gave an F_1 that was completely eliminated during the test of survival of the winter, and that F_2 showed a result suggesting the survival of the recessive extractives of the normal type. Further crossings of these have confirmed this original finding and extended the information as to the behavior of the alteration in inheritance, thus showing that the change between the conditions of the populations at Chicago and Tucson is of a gametic nature, and not somatic or temporary.

All tests of the heritability of this modification have been made with the sixth or later generations, for the reason that the alteration was apparently not at its full development until then, and in that the only means of distinguishing between the two conditions has been the survival test under the winter conditions at Chicago, earlier generations in which there were survivals of this test, would have given only results that had an error in them not possible of estimation. After the sixth generation at Tucson, all the cultures have thus far given the uniform result in crossings with the normal at Chicago, regardless of the direction of the cross, and these summated results are given in Table IX.

TABLE IX.

F_1 Populations.	Pairs.	Population.	Total Tested.	Hibernated.	Emerged.	% Eliminated.		Survived.
						Before.	During.	
Pairs: T. 99×C. 100; C. 100×T. 100; T. 99×C. 99; C. 99×T. 99	42	7	2,987	2,651	0	11.25±	88.75±	0
F_2 population.........	65	11	6,411	5,814	1,573	9.3 ±	66.1 ±	24.4±

In making these tests of the heritability of the alteration in the Tucson cultures, the F_1 series were all from matings of the first summer generation, whose progeny would hibernate, and for the F_2 tests, the over-wintering generations at Tucson and Chicago were crossed at Chicago, and the second summer generation tested. The results show in all that the F_1 population are in no instance able to survive the test, that the condition of the Tucson cultures is dominant, but that there is a segregation in F_2 and a survival from the test that is for the total series 24.4 per cent., about the expected proportion of the extracted recessives. These in breeding in F_3 show no indication of the Tucson condition, either in pairs or mass cultures, all surviving the hibernation test in F_4 in all respects like the normal Chicago materials.

The behavior in inheritance shows, therefore, no points of interest aside from the fact of its heritability, and the fact that a condition that has arisen rapidly, but apparently progressively, behaves in crossing when fully established as a Mendelian

dominant, but its behavior in a Mendelian way is no indication of its origin after its production.

Nature of the Alteration.

As already shown, the alteration in the Tucson cultures is physiological, involving a specific aspect of the reproductive cycle, that of the resting period which is passed in hibernation, and the only sure test of its existence is that of the survival under the conditions of the northern winter, at the place of origin of the stocks. This fact limits the possibilities of testing and experiments with the modifications that are possible, since no certain structural alterations in correlation with this change are demonstrated up to the present, and the differences in the color in the materials are too insecure criteria to be used at present.

What the physical or chemical changes are there is no indication at the present time, and one interpretation has about the same chances of being the correct one as any other excepting for the fact that the modified materials show a difficulty or slowness in water loss which seems to me to suggest more probably some altered condition in the colloids.

The chief question that concerns us in the consideration of the nature of the alteration, is whether it is an added or new condition, or the revival of an ancestral one.

The species *L. decem-lineata*, originally confined to the eastern slopes of the Rocky Mountain uplift, and eastward over the Great Plains into western Kansas and Nebraska, from whence it gradually spread eastward to the Atlantic coast, has as its nearest relatives, a series of species that are confined to the high plateaus of Mexico; areas of semi-desert with arid conditions, especially in the resting season. Using the usual criteria of kinship, similarity in structures and habits, the species *L. multitæniata* and *oblongata* are the nearest relations of this northern species phylogenetically.

All three species interbreed rather freely, are in developmental sequences similar, as in their ecological relations, in their reproductive cycles, in relation to the growing season of the habitat in which they live. These relations and conditions I have

recorded in a previous publication, along with the probable relations and phyletic history and relations of these organisms.

Both of the southern species in all of the locations in which I have information concerning them, must encounter during their period of hibernation desiccation in differing degrees of intensity, from a rather low one on the Plateau of Ahnuac, to intense conditions in the deserts of San Luis Potosi, and in hibernation do not go deep in the soils, but remain in the upper layers, or near the surface, whereas *decem-lineata* in its range goes deep in the soil. Owing to the disturbed and impossible conditions in Mexico, since this condition was discovered in the Tucson cultures, it has been impossible to either obtain new materials from the Mexican locations, or make desirable observations and tests that might have been made had more happy conditions prevailed. Fortunately some experiments made in 1908 and 1909 for other purposes have given data as a by-product that is of interest in this connection.

In both of these years crosses were made in the garden at Chicago of the C 100 stock and freshly obtained materials of both *L. oblongata* and *L. multitæniata*, the latter from Chapultepec near the City of Mexico, the former from Cuernavaca in the State of Morelos, Mexico. These were of the first summer generation in all species, and the F_1 populations were hibernated in the garden at Chicago under the full intensity of the winters of 1908–9 and 1909–10, with the results that are shown in Table X., all the materials being from the mating of pairs, and not from population cultures.

The experiences in the crossing of the three species shown in Table X. with the elimination of the hibernation F_1 population during the winter months is highly suggestive, and would give the basis for an interesting series of tests were it possible to obtain fresh materials from the Mexican locations. The complete dominance of the Mexican species over *decem-lineata* in the matter of survival in hibernation, indicates that in them the same or essentially the same condition exists as in the cultures at Tucson. Unfortunately, I have not had the necessary materials for the testing of their resistance to desiccation, and can therefore only conclude that the close identity in the survivals

from hibernation is due to the same causes as it is in the tests of the Tucson cultures. Unfortunately, I have no data concerning the survival of the Mexican types at Chicago that are at all comparable, so that I am not sure whether the pure Mexican

TABLE X.

		Pairs.	F1.		Hibernated.		Emerged.	
L. 10 lin. C. 100 ♂ × ♀ L. mult Chap.*	1908–09	7	170 ♂	196 ♀	147 ♂	171 ♀	0 ♂	1 ♀ died
	1909–10	14	278 ♂	304 ♀	229 ♂	258 ♀	2 ♂ died	1 ♀ died
" ♀ × ♂ "	1908–09	6	145 ♂	144 ♀	119 ♂	123 ♀	0 ♂	0 ♀
	1909–10	10	309 ♂	301 ♀	267 ♂	271 ♀	1 ♂ died	0 ♀
			902 ♂	945 ♀	762 ♂	823 ♀	3 ♂ died	2 ♀ died
L. 10 lin. C. 100 ♂ × ♀ L. obl. Cuer.†	1908–9	4	96 ♂	104 ♀	74 ♂	91 ♀	0 ♂	0 ♀
	1909–10	6	201 ♂	197 ♀	161 ♂	174 ♀	0 ♂	0 ♀
" ♀ × ♂ "	1908–9	5	131 ♂	127 ♀	100 ♂	93 ♀	0 ♂	0 ♀
	1909–10	9	246 ♂	265 ♀	197 ♂	213 ♀	0 ♂	0 ♀
			674 ♂	693 ♀	532 ♂	571 ♀	0 ♂	0 ♀
L. obl. Cuer. ♂ × ♀ L. mult. Chap.	1908–9	2	77 ♂	79 ♀	64 ♂	60 ♀	0 ♂	0 ♀
	1909–10	7	193 ♂	201 ♀	154 ♂	183 ♀	0 ♂	0 ♀
" ♀ × ♂ "	1908–9	3	66 ♂	73 ♀	60 ♂	64 ♀	0 ♂	0 ♀
	1909–10	4	107 ♂	119 ♀	91 ♂	99 ♀	0 ♂	0 ♀
			443 ♂	472 ♀	369 ♂	406 ♀	0 ♂	0 ♀

* Chapultepec. † Cuernavaca.

types are able to survive the Chicago winter or not, but it is my opinion that they would not.

The two Mexican species introduced into the deserts at Tucson showed in the first introductions in 1908 a huge elimination during hibernation, but they survived in the following year and continued a precarious existence for several years thereafter. Later introductions survived, or failed, for reasons not associated with the behavior in hibernation.

Upon the basis of the probable phylogenetic relations, the facts of the developmental cycles and the similarity in the ecological relations and needs, the data in the crossing of the species under the Chicago conditions and the survival values of the F_1 found therein, and the obvious recessiveness of the northern species in its hibernation behavior to the southern, gives the basis for several conclusions of interest as to the nature of the change.

(1) Assuming that the phylogenetic picture that we have drawn of the three species is essentially correct, and that *L. decem-lineata* is the younger derivative from the Mexican types, an essentially fair conclusion as far as conclusions in this line are at all possible, then the results of the tests show that there is a dominance of the older phylogenetic condition over the newer; or

(2) It may be concluded that in the origination of *L. decem-lineata* in its northern range, there had been a loss of an agent in the organization, which when present, produced retention and slow loss of water under desiccation, and its absence ease of loss, the experimental tests showing, therefore, the dominance of the presence over the absence. In that the reaction in crossing is a monohybrid one, one unit of difference may be assumed; or

(3) It may be concluded that we are dealing with a physical state in the living mechanism that exists as two conditions, alternative to one another in crossing reactions, but, which by the pressure of the medium may be altered to present first one aspect of its reaction, and under other conditions of the medium, the opposite aspect of its effect in the reactions of the living mechanism.

Which of the three possibilities is chosen as the correct one will depend entirely upon one's philosophical background, and from each a rather entertaining and plausible argument can be made. From purely mechanistic and physico-chemical considerations of living phenomena, I am inclined to the third possibility, as not only the more probable and thinkable, but also the one that as a working hypothesis offers the only opening for further experimental investigation.

In this preference I am no doubt prejudiced in my choice by many experiences with the behavior of the non-hibernating generation in which the behavior is easily, rapidly and positively altered, reversed at will by changes in the water content of the medium, producing reversibility of many of the trophisms of that generation. However, in this first summer generation, another condition of the reproductive cycle is under observation, and in that the reproductive cycle itself is present in two sharply alternative conditions, to breed or not to breed, activity or

hibernation, and further since these states are known in experiments to be alternative in hereditary behavior, one must not be too fast nor certain in arriving at conclusions in these complicated physiological activities.

Regardless of what one may believe concerning the alteration or the findings of subsequent investigations, certain aspects of its nature are demonstrated, its sharp alternativeness in heredity, its rôle in the determination of survival in different habitats, its directly adaptive nature; and its alteration in direct response to the conditions of the medium in adaptation to those conditions; these aspects of its nature show an alteration, a character capable of alteration, in ways that are of importance in the survival of the organisms, and that might be of decided value in evolutionary activities, speciation, and in ecological and distributional relations and results.

Method of Origin.

This account deals with experiments that are concerned with populations, and the results seem to be those of the alteration of the population as a whole. As shown, the experimental method was such that as far as the operator was aware, the choice of materials was random and entirely impersonal, and throughout any personal influence has been eliminated as far as known. Further, the living of the cultures in cages eliminated the action, if any, of enemies and further gave the cultures better conditions of life than they would have had in nature. Insofar as is known, therefore, the series deals with the response of an introduced population to the physical conditions of its environment. What are the methods of origin of this response and alteration? There are several possibilities:

1. That the original population consists of different pure lines, which under the conditions of the original habitat interbreed, and are hidden from recognition, but that under the Tucson conditions one has been able to survive with the characteristics found, and that the progressive development of its full intensity is the gradual elimination under the conditions of the experiment, of all but the one line with the characteristics finally developed. The conditions of experiment are, therefore, acting only as a sieve to separate out those capable of survival from those not

capable thereof, and we are determining in the experiment only some aspects of the composition of the population of the species in its original habitat.

2. That the introduced populations are uniform in composition, but that as the result of the altered conditions of life, variations are induced, of which only those whose reactions enable them to resist desiccation have survived; a natural selection of the fit by the physical environic factors.

3. That the population has latent within it the factor for water retention, and that this latency from an ancestral conditions under the conditions of the new habitat, is reactivated, mutates to the ancestral state in the materials of the experiments.

4. That the water relation in connection with hibernation is one due to some physico-chemical relation in the living colloids, that exists in one of two aspects, a condition of the substance, its type of manifestation depending upon the nature of the conditions of the medium.

There may be other possible methods whereby the condition is produced in the experiments, but these seem to me the most obvious possibilities.

That the results are due to the isolation under experiment from the introduced population of a pure line does not seem probable, or at all possible under the conditions of these experiments. All the basal populations introduced came from cultures in which the reproductive cycle was known to be homozygous with respect to the rhythm of the two generations, and since any heterozygousness in this rhythm is at once manifested and very obvious in the cultures, and was also repeatedly tested for in the stock cultures, and none found, the evidence is against the assumption. Further all the introduced stocks were taken from the original stocks on emergence from hibernation, after all the elimination incidental to hibernation had taken place. Were there lines in the original materials with the conditions that later developed in the Tucson cultures, which were isolated by the selective action of the Tucson experiments, and could not later survive the Chicago conditions when returned thereto; why, if present in the original stocks, were they not also eliminated by the conditions of the winter hibernation in the parental habitat?

Further in the elimination incident to hibernation, that found in preparation for hibernation remains about a constant percentage (about 11) throughout the series at Chicago and Tucson, the difference in the two is in the elimination that takes place during hibernation. The introduced populations at Tucson were all of individuals that had survived the Chicago winter's hibernation, whereas the materials returned to Tucson for testing did not survive the Chicago winter, as shown in the records of the tests.

There is, therefore, no evidence nor reason to interpret the results of the experiments as being due to the isolation at Tucson of pure lines from the introduced population, and every reason, as shown above, why this interpretation is highly improbable. The pure line concept is so facile and insidious in its possibilities for interpretation, that the result of the series of experiments are easily interpreted to conform therewith.

Since 1910 I have tried to develop pure lines from the materials at Chicago with respect to this water retention, and have not up to the present the least evidence that such exist, nor has the slightest success attended my efforts along that line. I cannot go beyond the facts of experience and must, therefore, conclude that upon the basis of the evidence available, pure lines of the characters developed in the Tucson experiments do not exist in the parental materials, and that the results are not due to the sieve-like action of the Tucson environment upon the introduced population, and I believe that this conclusion is entirely justified upon the basis of facts at present available.

It may be assumed that the condition developed was in correlation with some other characteristic operative at breeding, and that introduction eliminated those not possessed of this trait, but in these introductions, as in the T 99 series, from actual observation the entire introduced population took part in the initial breeding, and nowhere have I found the least indication that there has been any such relation, although I have been upon the lookout for them.

That the results are the product of the selection of favorable or adapted variations surviving from among multifarious variations, regardless of size, a product of natural selection, is without

any confirmation. The chief change takes place between introduction and the first period of hibernation in the elimination during hibernation, and there is therefore no opportunity for the operation of such selective action, even though it is present in the following two or three hibernations. I have tried to devise tests of this point, thus far without any success, and rather extensive breeding of pedigree pairs from these cultures have thus far given only negative results in this line. While I admit its possibility and its value as a basis for argument or interpretation, I am still without any evidences at all that can be used either way concerning this possibility, and am of the opinion that it is not, in view of the findings, a plausible interpretation of the results.

Concerning the possibilities of the reactivation of latent agents, under the conditions of experiment, a plausible interpretation could be made, if the initial assumption is made that such latent agents exist, an assumption that might receive some support from the probable phylogenetic history of these animals. Were any such condition latent in these beetles, it would seem that some indication would have been found of it in the rather extensive experiments that have been in progress in the last ten years, especially those that have to do with the reproductive cycle and hibernation; but as far as I am aware, there has been no indication thereof, and finding of it would have been of considerable value in many of my experiments, and after all the finding of the latency would not in this instance be of much service in the interpretation of the results.

It seems to me that the most plausible interpretation of the results of these experiments is that we are dealing with a case of the response of the entire introduced population, of uniform composition and behavior, in a determinate variation in Darwin's sense. A population reaction to the conditions of a new environment in a characteristic that is capable of existence, and does exist in species now living in alternative conditions depending upon the environic constellation encountered. That the alteration is directly adaptive in its manifestation implies neither purpose nor use, only that in the relation of water in the tissues, mechanistic considerations show at once that only two possible

conditions can exist; to hold the water or to let go of it, and there are no other possibilities. In other words, the alteration is adaptive and directive in its response, because from the physical nature of the materials no other relations are physically possible.

A crucial question is, why has the reaction not been as easily reversible on return to Chicago conditions? In this lies interesting possibilities for investigations now in progress. Up to the present the reversibility of this action has not certainly been obtained, although I have no doubt but that if I can discover the proper relations between the internal mechansim of these beetles and their medium that the reverse alteration of the Tucson condition to that at Chicago will be found.

Whether the reversal ancestralward, or what may be assumed to be an ancestralward direction, is easier than progressively, is for the present is a matter of opinion, and I am unable to discover any physico-chemical reason why it should be so, but we know many reversible actions that go easily through one series of events in one direction, and with more difficulty in the reverse and through another series of operations. In these purely physical and chemical reactions, ancestral influence is not at all involved, and I see no reason to assume the influence of ancestry and phylogenetic relationship in living reactions of the same general physical type.

Evolutionary Significance.

In the evolution of populations, in their habitat adjustments as in their distributional phenomena, reactions, accommodations of this sort may well have played a by no means minor rôle, and the value of adjustments of the kind found in these introduced populations at Tucson in these operations in nature are at once too obvious to need mention. Physiological adaptations of many sorts may well have their origin and owe their diversification to operations of precisely this general type, the fact of their heritability or behavior Mendelianwise having no significance or indication of the method of origin. In fact this and other experiences with these animals through many years strongly inclines me to the conclusion, that Mendelian alternativeness is

no certain criterion that they arose discontinuously. Osborn has presented from the findings of the fossil record, certain facts concerning the phylogeny of characters in mammals that now behave Mendelianwise in crosses, but, which arose, as far as the data of paleontology can decide, by gradual and continuous modification, and not by mutative jumps, and further in that the Mendelian reactions concern only extremes or alternatives in the same quality of the organism, it may result that the entire Mendelian phenomena are but the expressions of characters whose manifestation is capable of easy and reversible manifestation.

The fact that this alteration is inheritàble at all, may to some, seem incomprehensible, but it never segregates, its behavior in heredity is always that of a mono-hybrid, and in all experiences it behaves as a property characteristic of the whole, and possibly involves, therefore, an alteration not of determiners in the usual sense, but an alteration of the colloidal matrix of the tissues, soma and germ, which we must regard not as different kinds of substance, but as different expressions of one and the same specific kind of living material.

From this series of experiments, operations that are important in population evolution, especially of introduced populations, are certainly discovered, and has served in my hands for an entering wedge into the experimental investigation of questions concerning the evolution of populations, an aspect of evolution of which there is extremely little experimental analysis or investigation. In nature and the diverse products of the evolution phenomena in nature, our classification, schemes of phylogeny, our experiences are with populations of diverse things, in associated relations, both living and non-living, and more intimate knowledge of, and a broader understanding of these population problems seems most necessary for further progress in evolution investigations.

As an example of the manner, and the change necessary, and its production in the alteration of an organism originally characteristic of a mesophytic habitat into one in all ways adjusted to the rigors of the Arizona deserts, of the nicety of adjustment, its promptness, and direct adaptive nature, these experiments

provide illuminating examples and the basis for continued investigation of the same general aspect of the evolution problems.

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